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Automatically monitoring of dietary effects on rumination and activity of finishing heifers

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1 **Running head: Rumination and activity of beef heifers**

2 **Title:** Automatically monitoring of dietary effects on rumination and activity of finishing
3 heifers

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8 **Abstract**

9 Rumination and activity behaviors are important welfare indexes in beef cattle housing. The
10 main objective of this study was to assess if SCR automatic collars are able to reliably assess
11 the rumination and activity patterns of beef heifers. For this purpose, individual rumination
12 time and activity (RT and AT, respectively) were continuously recorded using an automatic
13 neck collar system (Hr-Tag, SCR Engineers Ltd, Israel) on the three trials performed. For the
14 Experiment 1, 60 Italian crossbred heifers were randomly assigned to one of two
15 experimental diets for 9 months: the CORN-SILAGE diet (CS), which included 50% forage
16 on dry matter (DM) basis (43% corn silage, 7% wheat straw) and the hay diet (HAY), with
17 57% forage on DM basis (28.5% grass hay, 28.5% alfalfa hay). Heifers consuming HAY diet
18 showed greater ($P < 0.05$) RT (min/day) and AT (bits/day), compared to CS diet. RT per kg
19 of Dry Matter and per kg of aNDFom intake were similar in the two experimental groups,
20 while RT per kg of peNDF intake was greater ($P < 0.05$) in the CS group compared to the
21 HAY one. Daily rumination and activity trends were significantly different among CS and
22 HAY group. In the second experiment 16 beef heifers were randomly allocated in two
23 homogeneous pens, containing 8 animals each one, and two non-homogeneous ones, where
24 16 animals were placed in more time steps, with different stocking density. The AT of non-
25 homogeneous pens was significantly higher respect to the homogeneous ones, suggesting a

26 distress condition for values higher than 309 bits/day AT. In the third experiment RT and AT
27 of 3 animals with respiratory disease were collected using the SCR system and compared
28 with AT and RT of the health animals. Sick animals presented significantly lower RT and
29 higher AT than the health ones. The cut-off to distinguish sick from health heifers were set to
30 537 bits/day AT and 381 min/day RT. In conclusion, this study demonstrated that SCR
31 automatic collars can reliably monitor different rumination and activity behaviors of beef
32 animals in various management conditions and different health status.

33

34 **Introduction**

35 In recent years, multiple devices have been developed and implemented by the dairy
36 industry to automatically monitor behavior and physiological parameters (Rutten et al., 2013;
37 Barkema et al., 2015), while few trials were made in beef cattle area. Physical activity levels
38 and rumination time are two parameters that are currently available for monitoring dairy cow
39 health, and it has already been demonstrated that rumination and activity alterations could be
40 associated to oestrus and to clinical and subclinical health disorders (Calamari et al., 2014;
41 Gáspárdy et al., 2014; Stangaferro et al., 2016). Monitoring activity combined with other
42 variable detections has been used to characterize pre-partum behavior, and predict calving in
43 dairy cows (Borchers et al., 2017). In addition, the automatically recording of activity and
44 rumination, and their correlation with animal performance, were assessed to measure the
45 effects of different rations on ruminal function (Adin et al., 2009) and to detect early onset of
46 lameness (Van Hertem et al., 2013) and mastitis (Stangaferro et al., 2016). Nevertheless, the
47 use of automatic and continuous monitoring of rumination and activity time is not yet
48 commonly used in beef farms. Rations used in finishing cattle are commonly characterized
49 by a low content of forages and a high use of concentrates in order to increase the energy
50 density of the diet and consequently the weight daily gain. In these conditions the rumination

51 behavior should be impaired; the possibility to record continuously this parameter could help
52 to detect easily and precisely the single sick animal inside of the group. Continuous
53 monitoring of behavioural and physiological parameters can aid early detection of sick
54 animals, allowing immediate and targeted therapy (Weary et al., 2009).

55 The automatic collar system (Hi-Tag; SCR Engineers Ltd., Netanya, Israel) is based
56 on the acoustics signals of rumination, thanks to a sensory microphone able to detect any
57 passage of feed bolus. In addition, the collars also continuously measure activity, which is
58 defined as presence or absence of head/neck movements (Schirmann et al., 2009; Schirmann
59 et al., 2013; Gentry et al., 2016). Rumination collars (SCR tag) have recently been used in
60 beef cattle in order to study the relationship between different dietary treatments, digestion,
61 rumination and activity behaviors (Gentry et al., 2016; Weiss et al., 2017). A recent study of
62 Marchesini et al., (2018) used rumination and activity data as health status and performance
63 indicators in beef cattle during the early fattening period.

64 Further studies are necessary to better know the rumination and activity patterns in
65 finishing beef cattle housed in different conditions. The objective of this study was to
66 monitor rumination and activity of finishing heifers underwent two different dietary
67 treatment (Experiment 1) and allocated either in homogeneous or non-homogenous pens
68 (Experiment 2). In addition, rumination and activity patterns of heifers in different health
69 status were studied (Experiment 3).

70

71

72 **Materials and Methods**

73 The Scientific Ethic Committee on Animal Experimentation of the University of
74 Bologna examined and approved the experimental protocol (n.: 4783-X/10 All: 17) used in
75 this study.

76 **Experiment 1.**

77 **For the first trial**, sixty Italian crossbreed heifers were divided in two homogenous
78 experimental groups, with similar characteristics of age (approximately 7 months) and body
79 weight (BW) (200.9 ± 30.6 kg, mean \pm SD). **After an 8-weeks adaptation period**, animals
80 were randomly allocated to 6 pens, each containing 10 heifers (space available/animal = 4.5
81 m²), located in the same housing facility. Heifers were individually weighed at the beginning
82 and at the end of the trial, in order to calculate average daily gain (ADG) and the gain to feed
83 ratio (G:F). Pens were then randomly assigned to 1 of 2 treatments (Table 1). Heifers were
84 fed once per day at 07:30 in the morning and received *ad libitum* diet based on a specific
85 amount of DM delivered, in order to have no residues in the manger one hour before the next
86 delivery. Access to fresh water was allowed continuously. Diets were prepared as total mixed
87 rations (TMR) with a horizontal mixer wagon (Zago 13-m³, ZAGO srl, PD, Italy) equipped
88 with a weighing scale. The corn-silage group (CS) received a basal diet composed of corn
89 silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%) and a
90 protein supplement (6%). The HAY group received a hay diet, based on higher percentage of
91 roughage, composed of alfalfa hay (28.5%) and grass hay (28.5%). Moreover, the diet
92 included earlage (28.5%), wheat hulls (9.5%), and a protein premix (5%). Animals were
93 slaughtered by pen, at an average BW of 426.74 ± 36.4 kg, following the standard weight
94 slaughtering procedures. Individual slaughter weights were taken in the abattoir immediately
95 before the slaughtering procedure. Harvest was completed at the local abattoir in the
96 Department of Veterinary Medical Science of Bologna in 1 wk period.

97 The individual rumination time (RT) and activity time (AT) were continuously
98 recorded during the last 90-days of the trial, for both groups of animals, using an automatic
99 neck collar system (Hr-Tag, SCR Engineers Ltd, Netanya, Israel). Animals were checked
100 daily to verify the right collar position. Data from individual tags were transferred to the
101 system software (Dataflow, SCR Dairy) automatically every 20 min *via antenna* located in
102 the barn. Rumination and activity time (RT and AT, respectively) were recorded by the
103 software as daily means (rumination minutes or activity bits during a day) and as 2h intervals
104 in order to study daily trends.

105 *Feed analysis*

106 TMR samples were collected at the beginning of experimental period, and then twice
107 a month. Feed samples were dried in a forced air oven (M700-VF, MPM instrument,
108 Bernareggio, IT) at 60°C for 48 h to determine DM content. Particle size distribution of the
109 dry diets was determined using a Penn State Particle Separator (PSPS. Mertens, 1997;
110 Heinrichs, 2013), and peNDF was calculated by multiplying the percentage of particles
111 retained above a 4 mm screen times the aNDFom content. For chemical analysis, dried diets
112 were ground separately in a Cyclone mill (1-mm screen; model SM100; Resch GmbH, Haan,
113 Germany). Feed samples were analyzed for ash, determined after 3 h combustion at 550°C in
114 a muffle furnace (Vulcan 3-550, Dentsply Neytech, Burlington, NJ); aNDFom (Mertens,
115 2002; Palmonari et al., 2017); acid detergent fiber (ADF); acid detergent lignin (ADL), and
116 crude protein (CP) (AOAC, 1990).

117 *Diet Characteristics and Fiber Particle Size*

118 Chemical composition of the two experimental diets is described in Table 1. The
119 percentages of CP, aNDFom, ADF in HAY diet were higher than in CS diet, while the CS
120 diet had a higher starch content. Particles separation are presented in Table 2. The proportion
121 of particles that were retained on the top of 19 mm and 8 mm sieves was greatest ($P < 0.05$)

122 for HAY diet, while the CS diet contained the greatest proportion ($P < 0.05$) of small
123 particles (< 4 mm) compared with HAY one. The physical effectiveness factor, given by
124 particles longer than 4 mm (sum of particles retained on the top 3 sieves of the PSPS) and
125 estimated peNDF were greater ($P < 0.05$) for the HAY diet compared to the CS one.

126

127 **Experiment 2. Homogeneous and non-homogeneous groups**

128 In the second experiment 32 animals were housed for 6 months in four pens in order
129 to study activity and rumination behavior under different environmental conditions. Sixteen
130 heifers were randomly allocated in two homogeneous pens (for age and BW), containing 8
131 animals each one; animals were divided in two homogeneous groups for age (approximately
132 7 months) and BW (200.9 ± 30.6 kg, mean \pm SD). Other sixteen heifers were randomly
133 allocated in different times in two non-homogeneous pens, with different stocking density.
134 Animals were placed in these two boxes in more steps: at the beginning of the experiment
135 (t1), 3 during the second month (t2) and during the fifth month (t3) of the trial. All 32
136 animals from homogeneous and non-homogeneous pens were fed with the same diet (the
137 CTR one). During the second trial, individual rumination time (RT) and activity time (AT)
138 were continuously recorded for all four groups of animals using the automatic neck collar
139 system (Hr-Tag, SCR Engineers Ltd, Netanya, Israel).

140 **Experiment 3. Detection of health status**

141 In the third experiment, rumination and activity behaviours of 3 beef cattle with
142 respiratory disease (sick animals) were collected using the SCR system and compared with
143 AT and RT of healthy animals (of the experiment 1). RT and AT of sick animals were
144 continuously recorded until the death of the animal, occurred for the symptoms worsening.

145 *Statistical analysis*

146 Data were analyzed by R version 3.4.0 (R Core Team). After checking for normal
147 distribution, chemical and physical composition of diets were analyzed by a t-Student test.
148 Pen was used as the experimental unit for the Experiment 1, while animal was used as the
149 experimental unit for the Experiment 2. and 3. Regarding the experiment 1, data concerning
150 animal performance (ADG, G:F, slaughter weight and age) were analyzed by the mixed
151 models including treatment (diet) as the fixed effect and the initial weight as the covariate.
152 Rumination and activity behaviors were analyzed by the mixed models. To analyze daily
153 rumination time (expressed as min/day, min/kg DM, min/kg aNDFom and min/kg peNDF)
154 and daily activity (bits/day) the fixed effects of treatment (diet), day, and their interactions
155 were included in the initial models. Pen was considered a random effect. Rumination and
156 activity raw data (min/2h and bits/2h, respectively) were analyzed by the mixed models
157 followed by the Tukey *post hoc* test for multiple comparison. The initial model included the
158 fixed effects of treatment (diet), time (2h) and their interactions. Pen was considered a
159 random effect. The final model for each parameter of interest was selected by backward
160 elimination of explanatory variables with $P > 0.05$. Data are expressed as least square mean
161 (LSM) and the standard error of the LSM (SEM). An effect was considered significant at $P <$
162 0.05.

163 As regards the experiments 2 and 3, the animal was considered the statistical unit.
164 Receiver Operating Characteristic (ROC) curves were generated to discriminate
165 homogeneous and non-homogenous groups (exp. 2) and to differentiate sick from healthy
166 animals (exp. 3), using RT and AT cut-off. To analyze daily RT and AT of homogeneous and
167 non-homogeneous pens (exp. 2), pen and period (t1, t2 and t3) were included in a mixed
168 model as fixed effects, while animal by day was considered the random effect. RT and AT of
169 sick and health animals (exp. 3) were studied by a mixed model including the health status of
170 animal as fixed effect and the animal by day as random effects.

171

172 **Results**

173 **Experiment 1**

174 *Feedlot performance*

175 ADG, G:F, initial and slaughter BW, housing period, and the age at slaughter of
176 feedlot cattle are presented in Table 3. No significant differences were observed for length of
177 housing period and age at slaughter between the two experimental groups. The CS group had
178 a higher slaughter BW and ADG ($P < 0.05$) than the HAY one. G:F was lower ($P < 0.05$) in
179 HAY heifers compared to CS group.

180 *Rumination and Activity*

181 The daily RT and AT were significantly ($P < 0.05$) different between the two
182 experimental groups. As shown in Fig. 1, daily RT of HAY group was significantly ($P <$
183 0.05) higher than that observed in the CS group (473.75 vs 405.22 min/day, SEM = 3.90,
184 respectively). A significant difference was also observed for daily AT, which was higher in
185 the HAY group compared to the CS one (619.31 vs 553.12 bits/day, respectively, SEM =
186 5.27). Significant differences ($P < 0.05$) were observed for rumination time per kg of DM and
187 peNDF intake, while there were no differences in rumination time per kg of aNDFom (Table
188 4).

189 Considering RT throughout the day (min/2h), significant differences were observed
190 within treatment, and between the two experimental groups, as shown in Fig. 2. About 4 h
191 after feed delivery, the results demonstrated a significantly increase of RT (+15 min/2h on
192 average respect to 08:00 h) in both experimental groups (29.10 ± 10.0 min/2h in CS group
193 and 30.60 ± 9.14 min/2h in HAY one). Moreover, in HAY group rumination was maintained
194 significantly ($P < 0.01$) higher (29.60 ± 9.55 min/2h average) for the following 6 h (from
195 12:00 h to 18:00 h). On the other hand, a significant ($P < 0.01$) rumination decrease ($29.10 \pm$

196 10.0 to 19.75 ± 8.35 min/2h) resulted in CS group from 12:00 h to 16:00 h, followed by a
197 rapid increase ($P < 0.05$) in the next 2h interval (26.52 ± 10.82 min/2h at 18:00 h). RT
198 progressively increased during night time (from 20:00 h to 04:00 h in CS group, and from
199 18:00 h to 04:00 h in HAY group). Moreover, the increment (from 20:00 h to 04:00 h) was
200 significantly ($P < 0.01$) greater in HAY compared to CS group (43.66 ± 15.63 vs $51.77 \pm$
201 20.23 min/2h on average for CS and HAY group, respectively). A considerable ($P < 0.01$)
202 RT decrease was observed from 04:00 h to 08:00 h in both experimental groups, until the
203 next feed delivery (16.75 ± 8.88 min/2h and 15.04 ± 9.97 min/2h in CS and HAY groups,
204 respectively, at 08:00 h).

205 As regards the AT throughout the day (bits/2h), significant ($P < 0.01$) differences
206 were observed within treatment and between the two-dietary treatment (Fig. 3). In both
207 experimental groups, the activity was significantly ($P < 0.05$) lower during the night time
208 hours (average 38.23 ± 9.51 bits/2h and 36.03 ± 11.04 bits/2h in CS and HAY groups,
209 respectively, from 22:00 h to 04:00 h) and began to increase from 04:00 h. During the day
210 (from 10:00 to 20:00), the averaged AT was significantly ($P < 0.01$) higher in the HAY group
211 than in the CS one (52.52 ± 12.94 bits/2h and 69.88 ± 19.28 bits/2h for CS and HAY,
212 respectively).

213 **Experiment 2**

214 Figure 4 shows the activity trend of homogeneous and non-homogeneous pens. As
215 visualized on the Figure 4, AT suddenly increased after the introduction of new animals into
216 the pen, and remained higher than homogeneous pen, still the entrance of the other heifers,
217 which determined an additional increment of all animals AT. Indeed, significantly
218 differences ($P < 0.0001$) were observed on AT among homogeneous and non-homogeneous
219 groups (511.68 ± 78.67 vs 768.32 ± 193.82 , respectively). In addition, the CV was highest in
220 non-homogeneous pens (0.25 vs 0.15 in homogeneous ones) demonstrating a great AT

221 variability among heifers of this experimental group. Significant interactions ($P < 0.001$)
222 resulted among period and pen (Figure 5). As concerns non-homogeneous pens, significant
223 increments of AT from t1 to t2, and subsequently from t2 to t3, were observed; while a
224 significant reduction of AT, from t1 and t2 to t3, resulted in non-homogeneous pens. The
225 ROC curve (Fig. 5) showed the best AT cut-off differentiating homogeneous pens from non-
226 homogeneous ones at 609.50 bits/day (area under the curve 0.92; 95 % CI 0.91 to 0.93;
227 sensitivity 0.81; specificity 0.91). No significant differences were observed on RT between
228 the two experimental groups (471.93 ± 66.80 vs 474.35 ± 71.54).

229

230 **Experiment 3.**

231 RT was significantly ($P < 0.01$) lower in sick animals, compared to the healthy ones
232 (267.91 ± 81.01 vs 456.65 ± 94.69 , respectively). Significant ($P < 0.01$) differences on AT
233 data were observed among healthy and sick animals (518.92 ± 112.64 vs 580.61 ± 89.44).
234 The ROC showed the best AT cut-off, to differentiate sick from healthy heifers, at 537
235 bits/day (area under the curve 0.70; 95 % CI 0.64 to 0.76; sensitivity 0.85; specificity 0.48)
236 (Fig. 6). Regarding RT data shown in Figure 7, the best cut-off, to discriminate sick and
237 health animals, was set at 381 (area under the curve 0.93; 95 % CI 0.90 to 0.95; sensitivity
238 0.88; specificity 0.83).

239

240 **Discussion**

241 The aim of Experiment 1 was to validate SCR collars on beef animals, underwent two
242 different dietary treatments. Significant differences have been observed in daily RT
243 (min/day) between the two experimental groups (Table 4). RT was significantly higher in
244 HAY group rather than in the CS one. Whereas peNDF is defined as the portion of NDF that
245 requires further mastication to allow passage out of the rumen (Mertens, 1997), this

246 increment could be justified by the significantly higher percentage of peNDF in HAY diet in
247 respect to the CS one. However, the RT relative to peNDF is significantly lower in HAY
248 group than in CS one, suggesting that other variables in addition to peNDF may influence the
249 ruminal behavior.

250 RT in terms of min/d, in both experimental groups, was higher than what observed in
251 Gentry et al (2016) study, while was similar in terms of min/kg peNDF. Thus, the difference
252 in daily RT (min/day) between CS and HAY group was not enough to justify the
253 significantly higher amount of peNDF in HAY diet, which appeared less efficient than the
254 one in CS group. According to Mertens (2002), to maximize RT and ADG the dietary
255 peNDF value should be 15% of ration DM, with a range from 12 to 18%, while Fox and
256 Tedeschi (2002) recommend that feedlot diets contain 7 to 10% peNDF. peNDF was defined
257 by Mertens (2002) as the percentage of the NDF retained on a screen with 1.18 mm openings
258 after dry sieving. Thus, considering that peNDF calculated in our study was based on the
259 amount of fiber retained on the top of 4 mm sieve, the values that we obtained were higher
260 compared with the two recommendations: 17.01% and 24.75% in CS and HAY diet,
261 respectively. Indeed, we observed both in the CS and HAY groups lower ADG and G:F ratio
262 compared with other studies, such as Gentry et al. (2016). A linear decrease of RT per kg of
263 peNDF as particle size increased was also observed by (Beauchemin and Yang, 2005).
264 Moreover, in Gentry et al. (2016) study, RT appeared to be more sensitive to particle size
265 than to sieving techniques, and no differences in RT per kg of peNDF were observed.
266 Bonfante et al. (2016) study demonstrated that different peNDF percentages in the diets of
267 Holstein heifers influence rumination time, but have no effects on rumen temperature and
268 rumen pH, suggesting that other variables contribute to rumen physiology. In our study, the
269 presence of wheat straw in CS diet (as 7% of DM), may have caused the increase of RT per
270 kg of peNDF, which resulted significantly greater than in the HAY group. These results are

271 in line with Farmer et al. study (2014) which demonstrated that the addition of chopped
272 wheat straw to lactating cows diets resulted in greater time spent chewing and eating per kg
273 of peNDF consumed, while the time spent chewing, eating, and ruminating were not affected
274 by reducing dietary forage. Also Mertens et al. (1997) reported that straw elicited
275 approximately 1.74 times the chewing response of alfalfa or grass forage of similar long
276 particle size. The importance of forage type and quality was also highlighted by Fustini et al.
277 studies (2011, 2017), which demonstrated that straw and different alfalfa cuttings influence
278 ruminating behaviors as well as the DMI. These results suggest that further researches are
279 needed to compare measurement techniques to calculate the appropriate peNDF requirements
280 in beef finishing diets, and to study the effect of different forage sources and levels in the diet
281 on ruminal behavior.

282 The significant differences observed in RT per kg of DM could be explained by the
283 greater rumination time relative to the similar DMI. Rumination per kg of NDF was similar
284 between the two experimental groups. Other authors (Beauchemin and Yang, 2005; Gentry et
285 al., 2016) observed no differences in rumination per kg of NDF for dairy cow and beef cattle,
286 respectively, consuming different amount or various particle sizes of forage.

287 The diets used in our experiment also differ for CP and starch contents, which could
288 have an influence on ruminal behavior. Previous studies demonstrated indeed that variations
289 in intake of dietary fractions modulated rumination patterns (Byskov et al., 2015; Mendes et
290 al., 2015; Farsuni et al., 2017). Further studies are necessary to better evaluate the influence
291 of each dietary component on ruminal behavior in beef cattle. Considering RT and AT trends
292 throughout the day, significant differences were observed between the two experimental
293 groups, and during the day within treatment. Daily rumination trend was similar to what
294 observed by Gentry et al. (2016), but the time spent in rumination for each 2 h time block
295 was greater in the current study. As shown also by Gentry et al. (2016), a slight increment of

296 RT was observed about 4 h after feed delivery, followed by a great increase during the
297 nighttime in both experimental groups. From 08:00 h to 10:00 h the time spent in rumination
298 was maintained significantly lower respect to the rest of the day, probably because of the
299 restriction of TMR that induced the animal to eat immediately after the morning delivery, and
300 to considerably reduce their rumination activity for the next 2 h. Based on the current data, it
301 would be of great interest to evaluate rumen behavioral changes in a different feed regiment,
302 such as *ad libitum* intake. Furthermore, as reported by Cavallini et al. studies (2017, 2018)
303 the total daily rumination did not change, while daily rumination and feeding patterns (RT/2
304 h) changed in dairy cows fed on *ad libitum* intake. Moreover, the increase of RT from 4 h
305 after feed delivery and during nighttime was significantly higher in HAY group compared to
306 the CS one, which showed a more fluctuating trend.

307 The AT daily mean was significantly higher in HAY group than in CS one. Moreover,
308 as expected, daily AT (bits/2 h) was significantly lower during the nighttime in both
309 experimental groups, while it was significantly higher ($P= 0.01$) during the day in HAY
310 group compared to the CS one. To date, there are a few studies about beef cattle activity, and
311 none of them observed any differences in AT between different feeding groups (Gentry et al.,
312 2016).

313 Variations of activity parameter are difficult to explain, since activity represents the
314 sum of a range of different behaviours, including eating, drinking, walking and social
315 interactions (Weary et al., 2009). These peaks of activity within a day may coincide with the
316 number of episodes of feed intake each day, since bulls fed a high-concentrate diet spent less
317 time eating and took shorter meals than bulls fed more concentrate diets (DeVries et al.,
318 2005; Mialon et al., 2008)

319 The aim of the Experiment 2 was to set an AT cut-off in order to distinguish
320 homogenous from non-homogeneous pens. As largely demonstrated, the social context has a

321 major effect on behavior. The social relationships can be agonistic (dominant-subordinate) or
322 affiliative (sociability) (Neave et al., 2018). As demonstrated by Zobel et al. (2011), beef
323 heifers in confined housing systems adopt different social strategies to respond to highly
324 competitive feeding environment. In that study, measures of feeding behavior were used to
325 detect different animal strategies characterized by engaging in competition or avoidance of
326 agonistic interactions. In addition, Gutmann et al. (2015) study on dairy cows showed that
327 long-term familiarity had a stronger effect on the intensity of social relationships, i.e.
328 regarding investment of time and energy, than very recent shared experience. Thus, keeping
329 well-acquainted animals together may contribute to a stable inner structure of a herd and thus
330 promote animal welfare. According to our results, the average AT of non-homogeneous pen
331 significantly increased when unknown animals were added into a preformed group. On the
332 contrary, the average AT of homogeneous pen significantly decrease after three months of
333 housing (Figure 4). On one hand, the increment of AT on non-homogeneous pen may
334 indicate distress situations determined by the entrance of unknown animal, which probably
335 led to increase animal competition and group rearrangement. On the other hand, the long-
336 term social interactions between animals of homogeneous pens determined a greater animal
337 tranquility, which contributed to the decrease of the average AT. These different activity
338 trends between homogeneous and non-homogeneous pens demonstrated that environmental
339 changes cause different heifers responses and corroborate the formation of affiliative
340 relationships among individuals of gregarious species, as extensively demonstrated by other
341 authors (Plusquellec and Bouissou, 2001; Miranda-de la Lama and Mattiello, 2010; Raussi et
342 al., 2010). These results suggested that AT could be an interesting and valid indicator of
343 animal welfare, particularly when they are housed in confined systems. The automatically
344 monitoring of AT, could contribute to better understand social interactions between animals
345 and distress conditions. Through the ROC curve we were able to distinguish non-

346 homogeneous pens from the homogeneous one, defining an AT cut-off of 609.50 min/day.
347 The abnormal general activity of non-homogeneous pens was thus detected over this cut-off.
348 Further researches are needed to better investigate the relationships between general activity
349 and other variables, such as lying, feeding and ruminating behaviors, with the use of different
350 technologies, in order to understand the physiological or altered activity pattern of beef cattle.

351 The aim of Experiment 3 was to examine whether it was possible to identify sick beef
352 cattle through the continuous monitoring of activity and rumination. Daily rumination time
353 resulted significantly lower on sick animals, while there was an increment of the daily
354 activity time in the same group of animals. In our study it was not possible to register
355 rumination and activity patterns before and after the appearance of clinical signs, because the
356 three sick animals presented the clinical symptoms suddenly after the beginning of the
357 experiment and they all died before the trial end. Thus, we were able to identify RT and AT
358 cut-off in order to distinguish sick and healthy animals using the ROC curves. As regards RT,
359 great sensitivity and specificity were obtained at 381 min/day cut-off, whereas worse results
360 were obtained with an AT ROC curve. Indeed at 537 bits/day AT, corresponding to the
361 optimum cut-off designed by the curve, resulted a good sensitivity (0.85), but a low
362 specificity (0.48). This low value of specificity is not considered acceptable because of the
363 high number of false positives which had to be taken into account to avoid the unnecessary
364 use of antibiotics. In dairy cows changes in patterns of rumination and activity during *E.*
365 *Coli* mastitis (Stangaferro et al., 2016) and lameness (Van Hertem et al., 2013) permitted the
366 detection of affected animals before the appearance of clinical symptoms. A recent study of
367 Marchesini et al., (2018) on beef cattle during the early fattening period demonstrated that
368 activity and rumination monitoring allows the detection of bovine respiratory disease and
369 lameness. Conversely to the previous studies, our results showed that activity daily time was
370 higher in sick animals than in healthy ones, suggesting different temperament and probably a

371 more agitation of the sick heifers. In line with the previous studies, RT was significantly
372 affected by health animal status and the reduced RT in sick animals permitted to define a
373 valid cut-off (with good sensibility and good specificity) to predict animal disease.

374

375 **Conclusion**

376 In conclusion, this study demonstrated that RT and AT are important welfare indexes
377 in beef cattle housing. The rumination and the activity patterns of beef animals are modulated
378 by different parameters like: the housing management, the dietary treatment and the animal
379 stocking density. In addition, rumination and activity daily time are often affected by health
380 animal status. These results suggest that the automatic detection of these parameters could be
381 useful to monitor the animal welfare during the housing period and to study the physiological
382 rumination and activity patterns of beef cattle. Further studies are needed to correlate RT and
383 AT patterns to the other welfare indexes such as individual feeding behaviors, rumen activity
384 parameters and animal performance, in order to evaluate their relationship. In this way, the
385 routinely use of neck collar system can potentially help the herd management and optimize
386 the performance.

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391

392 **Conflicts of interest**

393 The authors declare no conflicts of interest.

394

395

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492 **Table 1.** Ingredients and chemical composition of corn-silage (CS) and HAY diets fed to
 493 beef cattle for a restricted intake; the diets were formulated to be different in forage content
 494 (evaluated as physical effectiveness factor and peNDF).

Item, % DM basis	Dietary treatment ¹	
	CS	HAY
Cornsilage	43	
Earlage	25	28.5
Grass hay	-	28.5
Alfalfa hay	-	28.5
Wheat hulls	10	9.5
Corn meal	9	
Wheat straw	7	-
Protein premix ²	6	5
Forage:Concentrate	50	57
<u>Calculated nutrient values, DM basis</u>		
DM (%)	55.25	72.5
CP (%)	10.67	14.15
Ash (%)	5.49	7.76
aNDFom (%) ³	36.06	44.85
ADF (%)	22.55	33.69
ADL (%)	5.58	5.81
Starch (%)	35.72	18.6

495
 496 ¹CS = corn silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw
 497 (7%) and protein premix (6%). HAY = alfalfa hay (28.5%) and hay (28.5%), earlage
 498 (28.5%), wheat hulls (9.5%) and protein premix (5%).

499 ²Premix protein of CS diet contained (as fed): crude protein 37.2%, ash 15%, cellulose 9%,
 500 crude fat and oils 3%, Ca 2.2%, Na 1.8%, P 0.8%, Mg 0.70%, Methionine 0.5%, Vit A (E
 501 672) 45.000 U.I., Vit D3 (E 671) 14.500 U.I., VIT E **3a700 115mg**. Sodium selenite (E 8)
 502 1971 mcg, Zinc sulphate monohydrate (E 6) 1644 mg, Manganous sulphate monohydrate (E
 503 5) 893 mg, Ferrous sulfate monohydrate (E 1) 304 mg, Cupric sulphate pentahydrate (E 4)
 504 275 mg, Potassium iodide (E 232) 32mg, Urea 40000 mg.

505 ³aNDFom = amylase- and sodium sulfite treated NDF, corrected for ash residue (Palmonari
 506 et al., 2017).

507

508 **Table 2.** Physical characteristics and particle size distribution (%) of the corn-silage (CS) and
 509 HAY diets fed to beef cattle for a restricted intake; the diets were formulated to be different
 510 in forage content (evaluated as physical effectiveness factor and peNDF)
 511

Item	Dietary treatment ¹			
	CS	HAY	SEM	P-value
No. of samples	18	18	-	-
aNDFom (%)	36.06	44.85	2.29	0.04
	Retained screen (%) ²			
Sieve screen size (mm)				
19.0	9.77	16.50	0.22	0.01
8.0	18.45	23.21	0.32	0.02
4.0	18.94	15.48	0.67	0.01
Pan (particle less than 4mm)	52.83	44.81	0.45	0.01
Physical effectiveness factor ³	47.17	55.19	5.60	0.01
Estimated peNDF ⁴ (% DM) ⁴	17.01	24.75	3.24	0.01

512
 513 ¹CS= corn silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw
 514 (7%) and protein premix (6%). HAY = alfalfa hay (28.5%) and grass hay (28.5%), earlage
 515 (28.5%), wheat hulls (9.5%) and protein premix (5%).

516 ²Particle size was measured using the PSPS (Heinrichs, 2013).

517 ³Physical effectiveness factor: the percentage of sample larger than 4 mm in particle size (top
 518 3 sieves)

519 ⁴Percent peNDF was estimated by multiplying the physical effectiveness factor by the
 520 percentage of aNDFom (as a decimal) of the ingredient before separation.

521 **Table 3**

522 Effect of two different dietary treatments (CS and HAY diets) on finishing heifer
 523 performance.

Item	Dietary treatment ¹		SEM	P- value
	CS	HAY		
n (pens of 10 heifers)	3	3		-
Initial BW (kg)	197.86	203.90	4.84	-
Initial age (day)	214	248	17.43	-
Housing period (day)	285.09	293.93	4.64	0.85
DMI (kg/day)	8.38	8.32	0.16	-
ADG (g /day)	836	731	0.02	0.04
G:F (g/kg)	100.38	87.99	2.80	0.03
Slaughter BW (kg)	442.52	408.96	5.75	0.04
Slaughter age (day)	481.32	489.27	16.80	0.87

524

525 ¹CS = corn silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw
 526 (7%) and protein premix (6%). HAY = alfalfa hay (28.5%) and HAY hay (28.5%), earlage
 527 (28.5%), wheat hulls (9.5%) and protein premix (5%).
 528

529 **Table 4.**

530 Effect of dietary treatments on rumination time per kg of DM, NDF and physically effective

531 NDF (peNDF) consumed.

Item	Dietary treatment ¹			P-value		
	CS	HAY	SEM	Treatment	Day	Int. ²
No. of observations (pens of 10 heifers)	3	3		-	-	-
Rumination (min/day)	401.88	481.83	5.73	0.01	<0.01	0.12
Rumination (min/kg)						
DM	47.96	57.91	2.10	<0.01	-	-
NDF	132.99	129.12	6.80	0.16	-	-
peNDF	281.97	233.97	8.37	<0.01	-	-

532

533 ¹CS = corn silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw
534 (7%) and protein premix (6%). HAY = alfalfa hay (28.5%) and grass hay (28.5%), earlage

535 (28.5%), wheat hulls (9.5%) and protein premix (5%).

536 ²Int = interaction between Treatment and Day factors

537

538

539 **Figure 1.**

540 Boxplots representing the daily rumination (min/24h) and activity (bits/24h) time in finishing
541 beef cattle subjected to two different diet treatments (CS and HAY groups). CS = corn silage
542 (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%) and protein
543 premix (6%). HAY = alfalfa hay (28.5%) and grass hay (28.5%), earlage (28.5%), wheat
544 hulls (9.5%) and protein premix (5%). Notches represent 95% CI. Asterisks mean significant
545 ($P < 0.05$) differences between the treatment groups.

546 **Figure 2.**

547 Effects of diets (CS and HAY) on rumination behavior (min/2h) in finishing beef feedlot. CS
548 = corn silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%)
549 and protein premix (6%). HAY = alfalfa hay (28.5%) and grass hay (28.5%), earlage
550 (28.5%), wheat hulls (9.5%) and protein premix (5%). The arrow represents the feeding at
551 07.30 h. $n = 6$ pens (3 for each treatment group). The error bars represent 95% CI. Different
552 letters mean significant differences between hours, within the same treatment (small letters
553 for the CS group and capital letters for the HAY one). Treatment ($P < 0.01$; SEM = 1.77).
554 Hour ($P < 0.01$; SEM = 0.16). Treatment x Hour ($P < 0.01$; SEM = 0.22).

555 **Figure 3.**

556 Effects of diets (CS and HAY) on activity behavior (bits/2h) in finishing beef feedlot. CS =
557 earlage (43%), corn silage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%) and
558 protein premix (6%). HAY = alfalfa hay (28.5%) and grass hay (28.5%), earlage (28.5%),
559 wheat hulls (9.5%) and protein premix (5%). The arrow represents the feeding at 07:30 h. $n =$
560 60 (30 for each treatment group). The error bars represent 95% CI. Different letters mean
561 significant differences between hours, within the same treatment (small letters for the CS
562 group and capital letters for the HAY one). Different letters mean significant differences

563 between hours, within the same treatment (CS or HAY diet). Treatment ($P = 0.61$; SEM=
564 4.95). Hour ($P < 0.01$; SEM= 0.14). Treatment x Hour ($P < 0.01$; SEM 0.21).

565 **Figure 4.**

566 Graphics represents the average daily AT of homogeneous and non-homogeneous pens
567 relative to housing period (t1, t2 and t3). The asterisks indicate significant differences among
568 groups for $P < 0.05$; different superscripts indicate significant differences among periods
569 within the group for $P < 0.05$.

570 **Figure 5.**

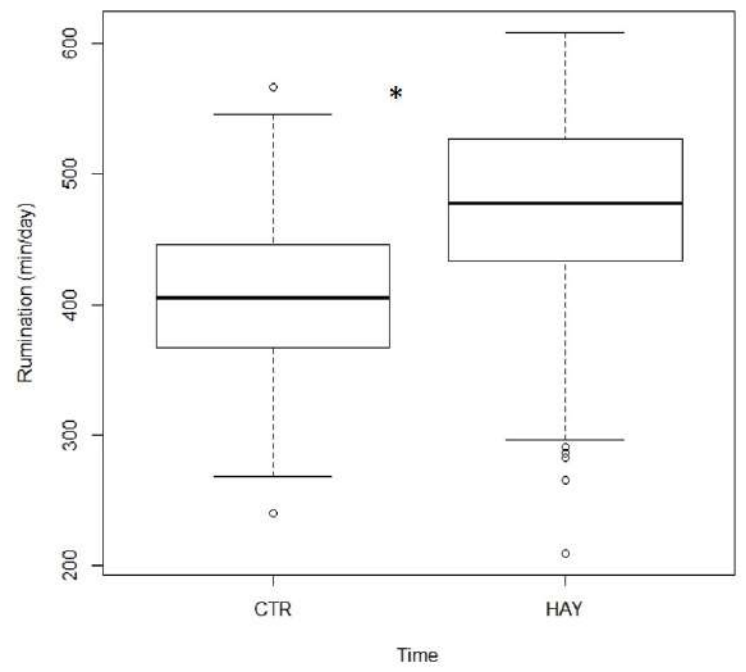
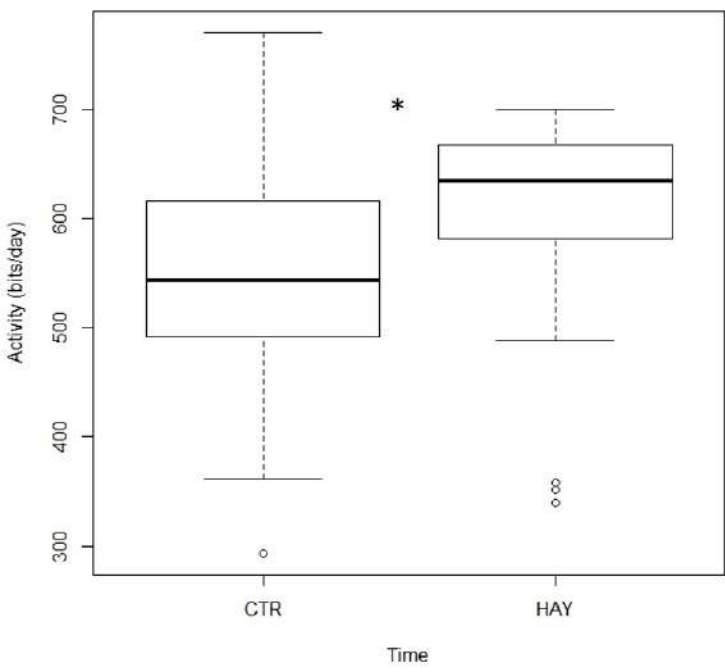
571 The receiver operator curve showed the best cut-off of AT at 609 bits/min, with a sensitivity
572 of 0.81 and a specificity of 0.91 (area under the curve: 0.92; 95% CI 0.91 to 0.93), to
573 distinguish homogeneous pens from non-homogeneous ones.

574 **Figure 6.**

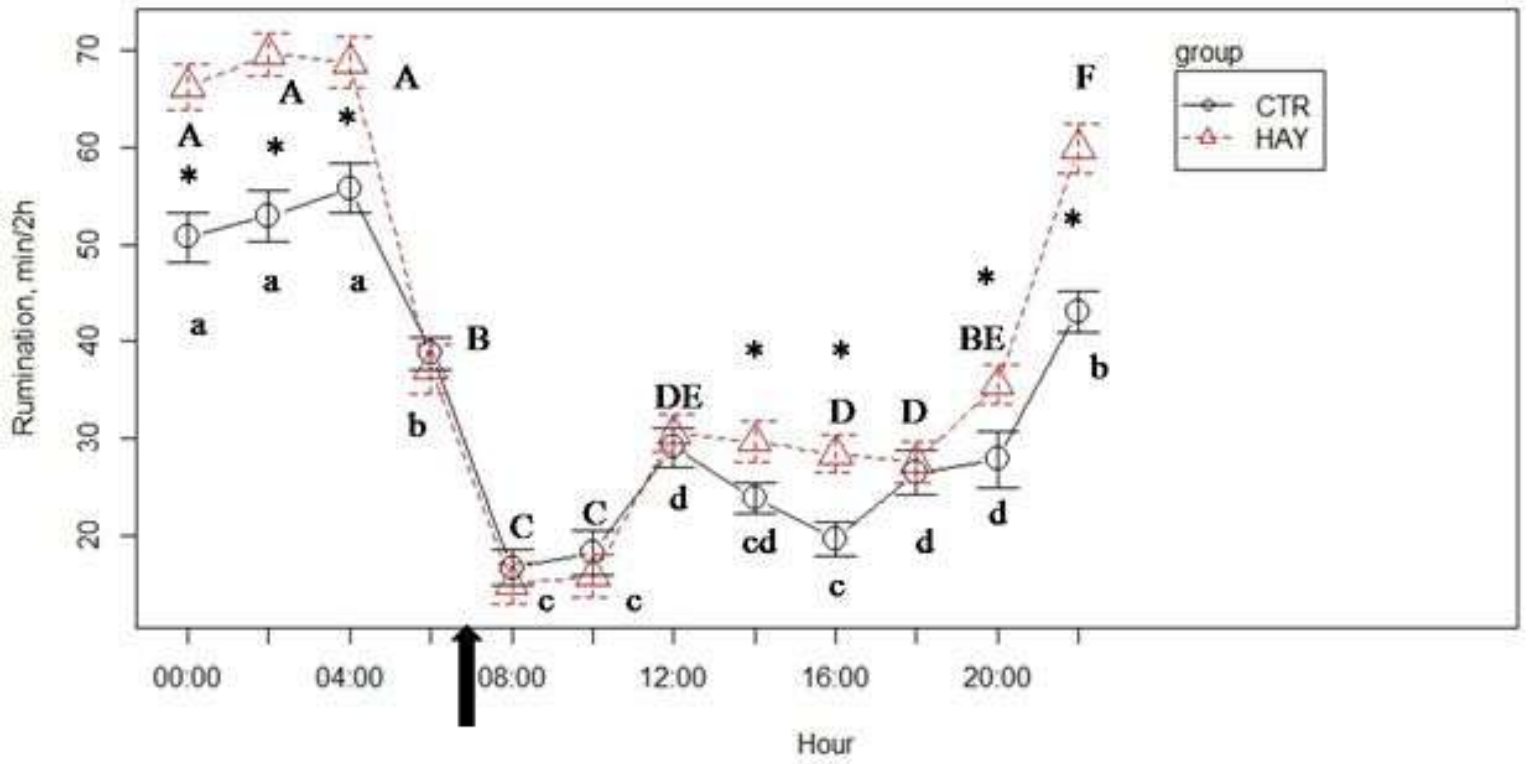
575 The receiver operator curve showed the best cut-off of AT at 537 bits/min, with a sensitivity
576 of 0.85 and a specificity of 0.48 (area under the curve: 0.70; 95% CI 0.64 to 0.76), to
577 distinguish sick animals from health ones.

578 **Figure 7.**

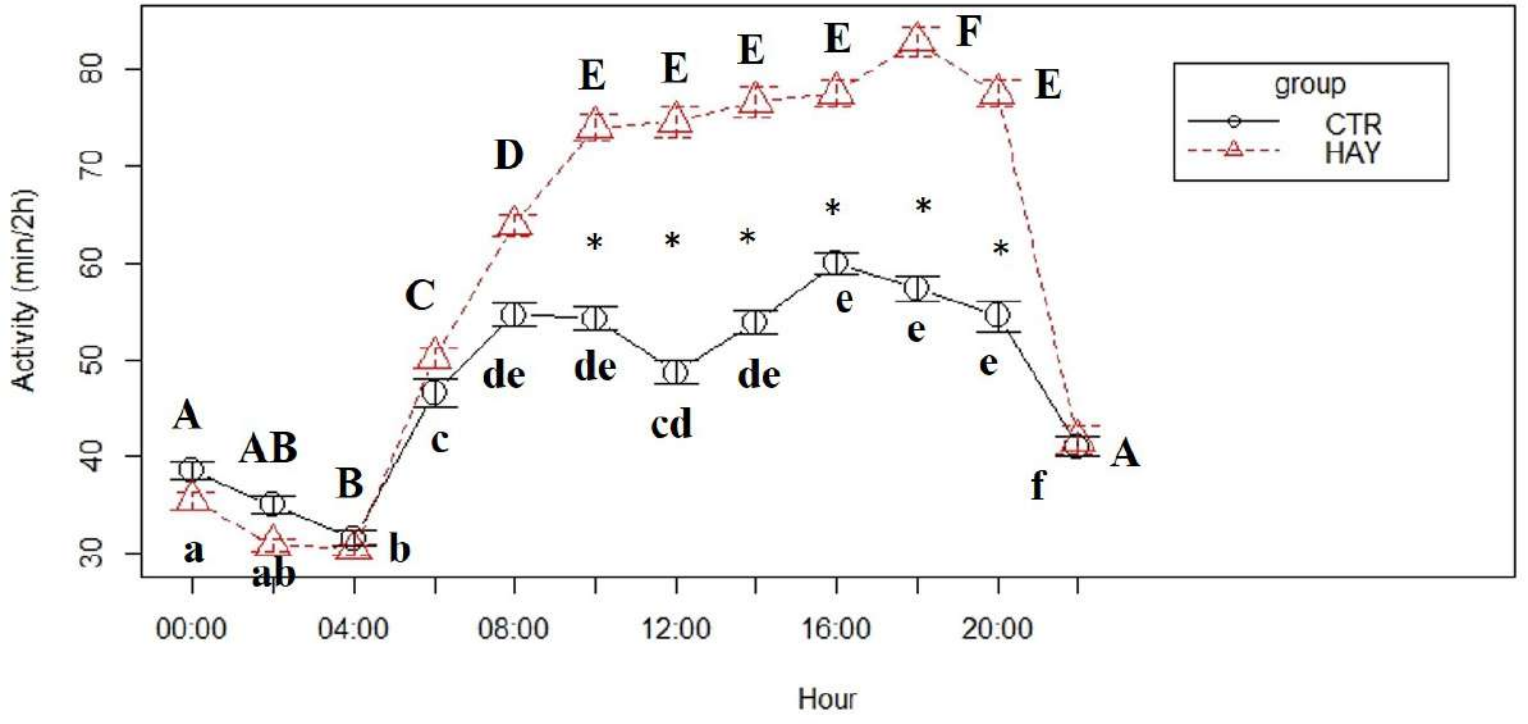
579 The receiver operator curve showed the best cut-off of RT at 381 bits/min, with a sensitivity
580 of 0.88 and a specificity of 0.83 (area under the curve: 0.90; 95% CI 0.93 to 0.95), to
581 distinguish sick animals from health ones.

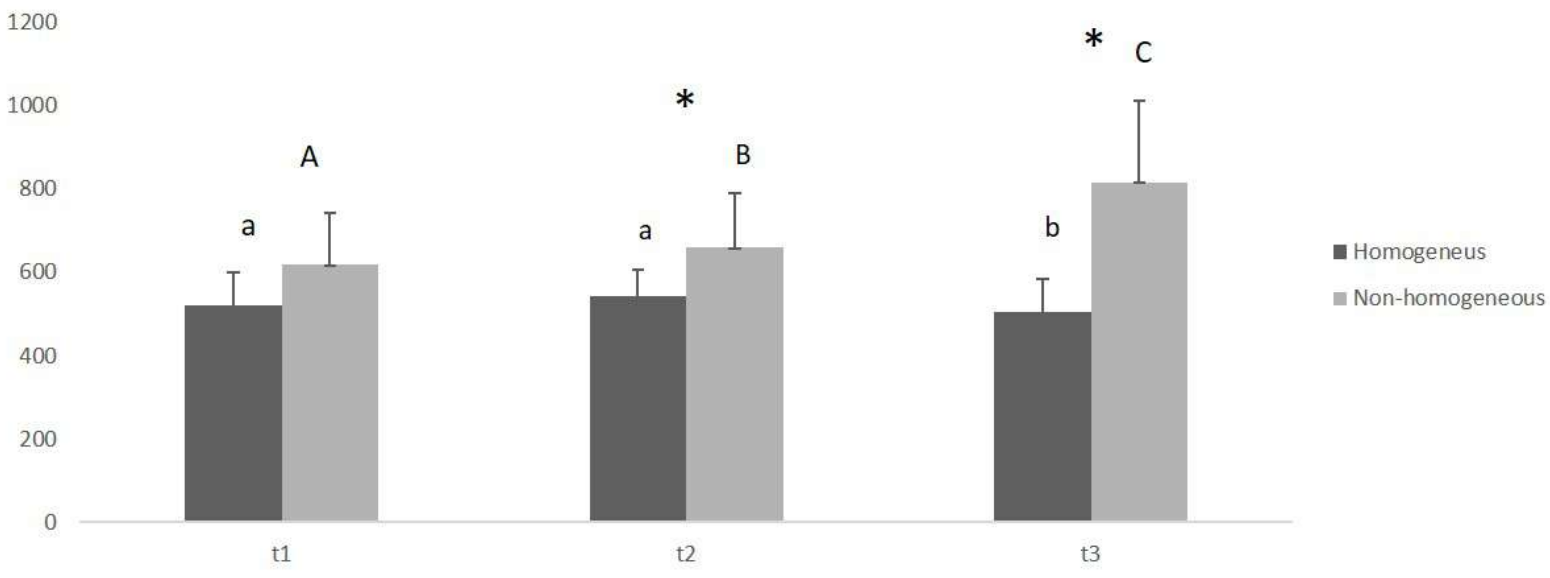


Rumination

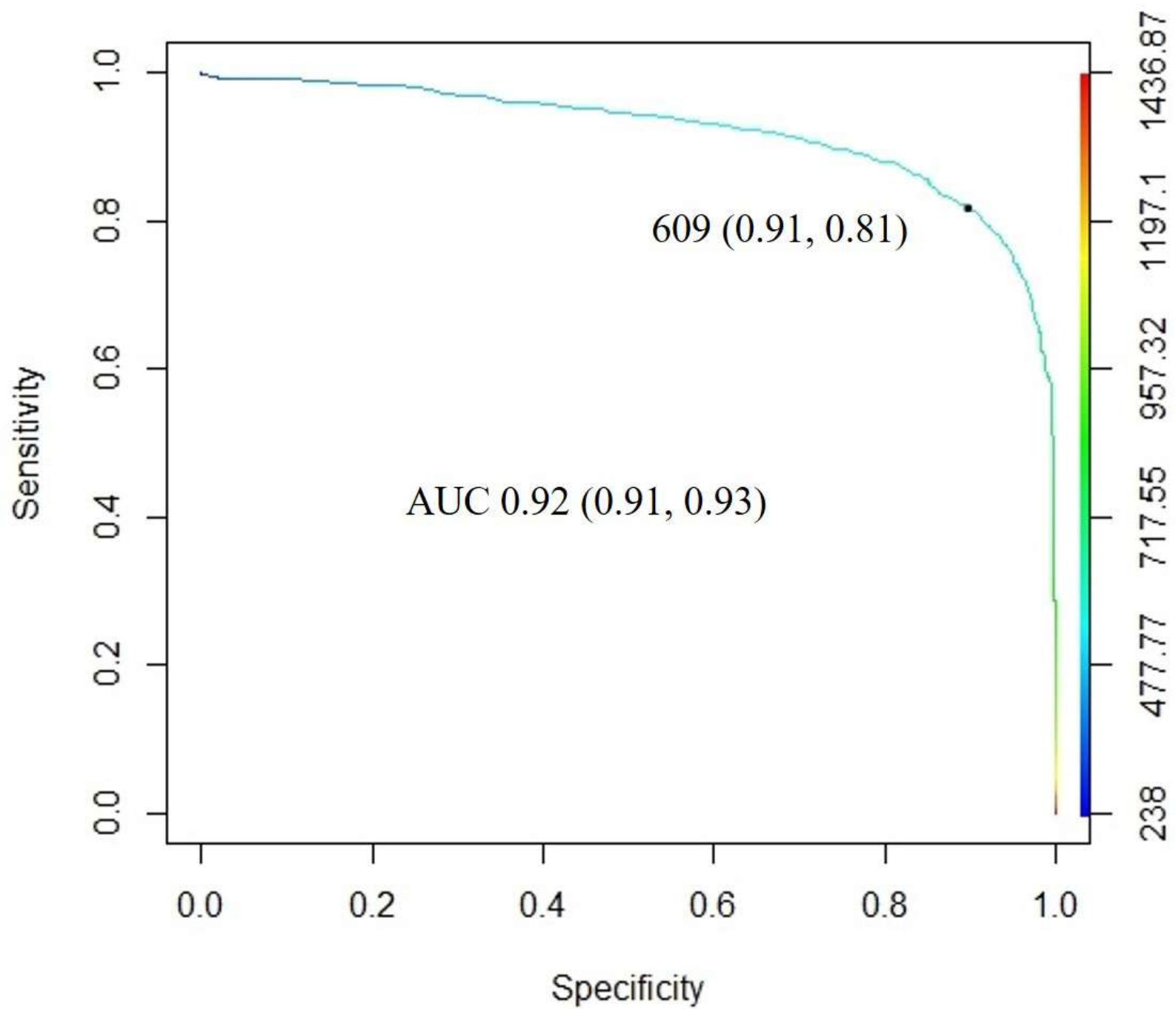


Activity

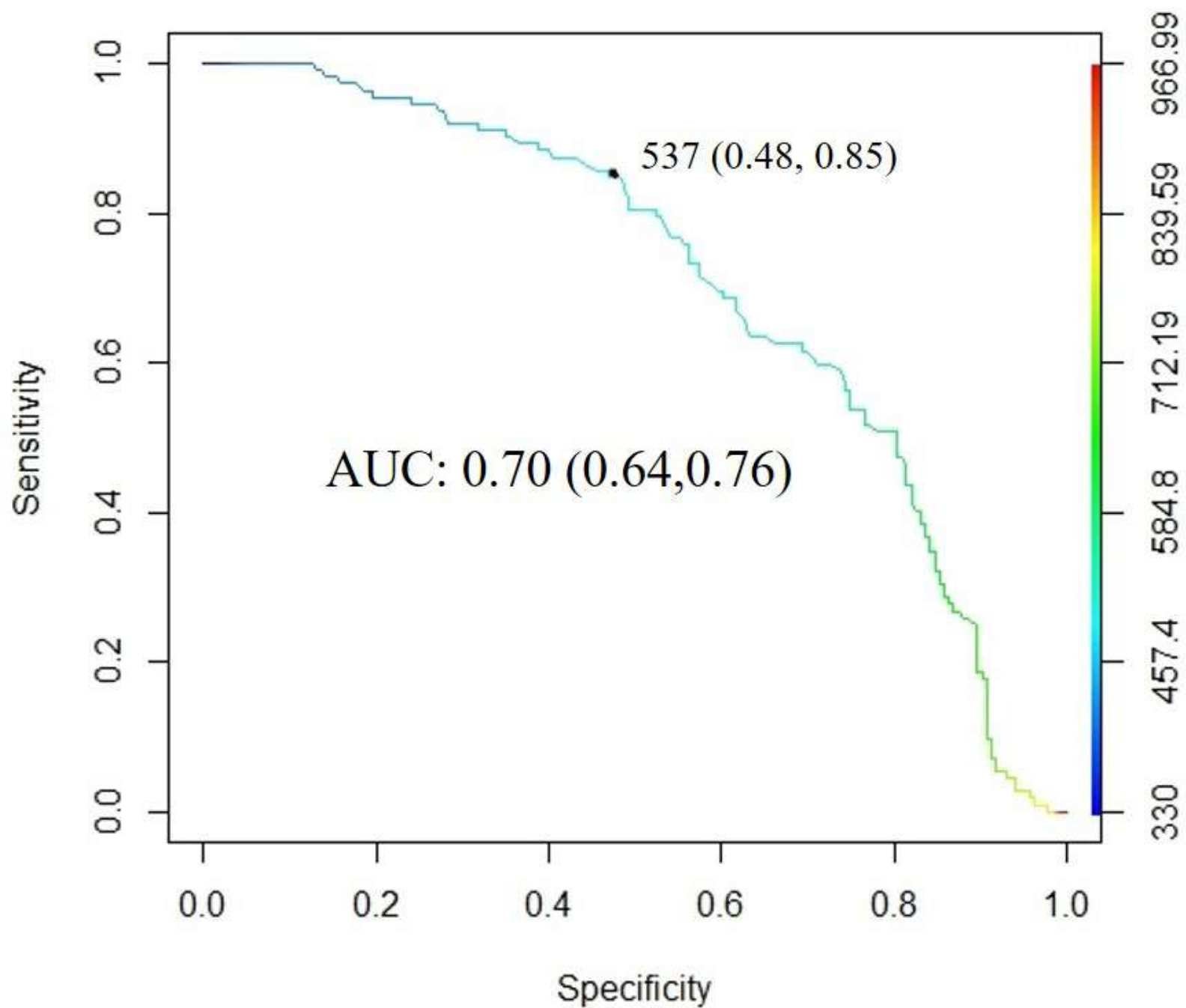




Activity



Activity



Rumination

